

Application No. 09/462,408
Attorney's Docket No. 020600-283
Page 2

AMENDMENTS TO THE CLAIMS:

This listing of claims will replace all prior versions, and listings, of claims in the application:

LISTING OF CLAIMS:

Claim 1. (Previously Presented) A method for characterizing DNA, which comprises:

- (i) providing a population of fragments of said DNA, each fragment having cleavably attached thereto a mass label for identifying a feature of that fragment;
- (ii) separating the fragments on the basis of their length by capillary electrophoresis, thereby determining the length of each fragment;
- (iii) cleaving each fragment in a mass spectrometer by collision to release its mass label; and
- (iv) determining each mass label by mass spectrometry to relate the feature of each fragment to the length of the fragment in order to characterize said DNA.

Claim 2. (Previously Presented) A method according to claim 1, which further comprises the following steps before step (i):

- (a) providing at least one DNA single-stranded template primed with a primer; and
- (b) generating the population of fragments of said DNA from the at least one template, wherein the population comprises at least one series of DNA fragments, the or each

Application No. 09/462,408
Attorney's Docket No. 020600-283
Page 3

series containing all possible lengths of a second strand of DNA complementary to the or each template;

wherein the feature of each fragment determined by each mass label relates to a nucleotide or nucleotide sequence at one end of each fragment, so that each nucleotide is related to a position in the template associated with the mass label so as to deduce the sequence of the or each template.

Claim 3. (Previously Presented) A method according to claim 2, wherein the series of DNA fragments is provided by contacting the template in the presence of DNA polymerase with a mixture of nucleotides for hybridising to the template for forming a second strand of DNA complementary to the template, wherein the mixture further comprises a set of four probes containing all four nucleotides for hybridising to the template in which the nucleotide of each probe comprises a modified nucleotide which is capable of polymerising to the second strand of DNA but blocked to prevent further polymerisation thereto and which is cleavably attached to the mass label, which mass label is uniquely resolvable in mass spectrometry for identifying the modified nucleotide, and wherein each fragment is terminated with one of the probes.

Claim 4. (Previously Presented) A method according to claim 2, wherein the at least one template is a plurality of templates and the series of DNA fragments is provided by contacting each template in a separate reaction vessel in the presence of DNA polymerase

Application No. 09/462,408
Attorney's Docket No. 020600-283
Page 4

with a mixture of nucleotides for hybridising to the template for forming a second strand of DNA complementary to the template, wherein the mixture further comprises a set of four probes containing all four nucleotides for hybridising to the template in which the nucleotide of each probe comprises a modified nucleotide which is capable of polymerising to the second strand of DNA but blocked to prevent further polymerisation thereto and which is cleavably attached to the mass label, which mass label is uniquely resolvable in mass spectrometry for identifying the modified nucleotide, wherein each fragment is terminated with one of the probes, and wherein each set of mass labels from each set of four probes associated with each reaction vessel is different from the other sets of mass labels; and the fragments are pooled before step (ii).

Claim 5. (Previously Presented) A method according to claim 2, wherein the at least one template is a plurality of templates and the series of DNA fragments is provided by contacting each template in a separate reaction vessel in the presence of DNA polymerase with a mixture of nucleotides for hybridising to the template for forming a second strand of DNA complementary to the template, wherein the mixture further comprises a probe containing only one of the four nucleotides for hybridising to the template, the nucleotide of which probe comprises a modified nucleotide which is capable of polymerising to the second strand of DNA but blocked to prevent further polymerisation thereto, wherein each fragment is terminated with the probe and wherein either the primer or the modified nucleotide of the probe is cleavably attached to the mass label, which mass label is associated with the reaction

Application No. 09/462,408
Attorney's Docket No. 020600-283
Page 5

vessel and uniquely resolvable in mass spectrometry from the mass label in the other reaction vessels for identifying the modified nucleotide used in the reaction vessel and the fragments are pooled before step (ii).

Claim 6. (Previously Presented) A method according to claim 2, wherein the at least one template is a plurality of templates and the series of DNA fragments is provided by contacting the plurality of templates in each of four separate reaction vessels in the presence of DNA polymerase with a mixture of nucleotides for hybridising to the template for forming a second strand of DNA complementary to the template, wherein the mixture further comprises a probe containing in each of the reaction vessels only one of the four nucleotides for hybridising to the template, the nucleotide of which probe comprises a modified nucleotide which is capable of polymerising to the second strand of DNA but blocked to prevent further polymerisation thereto, wherein each fragment is terminated with the probe and wherein the primer is cleavably attached to the mass label, which mass label is associated with the primer and uniquely resolvable in mass spectrometry from the mass labels associated with the other primers used in the reaction zone; and wherein each nucleotide from its corresponding reaction vessel is related to its position in the template.

Claim 7. (Previously Presented) A method according to claim 2, wherein the at least one template is four sets of DNA single-stranded templates, each set comprising an identical plurality of DNA single-stranded templates and the series of DNA fragments is

Application No. 09/462,408
Attorney's Docket No. 020600-283
Page 6

provided by contacting each set in a separate reaction vessel in the presence of DNA polymerase with a mixture of nucleotides for hybridising to the templates for forming a second strand of DNA complementary thereto, wherein the mixture further comprises a probe containing in each of the reaction vessels only one of the four nucleotides for hybridising to the template, the nucleotide of which probe comprises a modified nucleotide which is capable of polymerising to the second strand of DNA but blocked to prevent further polymerization thereto, wherein each fragment is terminated with the probe and wherein each of the templates of the four sets is primed with a primer to which the mass label is cleavably attached, which mass label which uniquely resolvable in mass spectrometry from the mass labels corresponding to the other templates and which is relatable to its respective template and its respective reaction vessel wherein the fragments are pooled before step (ii), and each nucleotide from its corresponding reaction zone is related to its position in the template.

Claim 8. (Previously Presented) A method according to claim 2, wherein the at least one template is a plurality of templates and the series of DNA fragments is provided by contacting each set of templates in a separate reaction vessel in the presence of DNA polymerase with a mixture of nucleotides for hybridising to the templates for forming a second strand of DNA complementary thereto, wherein the mixture further comprises a set of four probes containing all four nucleotides for hybridising to the template in which the nucleotide of each probe comprises a modified nucleotide which is capable of polymerising to the second strand of DNA but blocked to prevent further polymerisation thereto and which

Application No. 09/462,408
Attorney's Docket No. 020600-283
Page 7

is cleavable attached to the mass label, which mass label is uniquely resolvable in mass spectrometry for identifying the modified nucleotide, wherein each fragment is terminated with one of the probes, and wherein each set of mass labels from each set of four probes associated with each reaction vessel is different from the other sets of mass labels and, before step (ii), the fragments are pooled and the pooled fragments are sorted according to a subsequence having a common length of 3 to 5 bases adjacent to the primer to form an array of groups of sorted fragments, wherein each group is spatially separated from the other groups.

Claim 9. (Previously Presented) A method according to claim 2, wherein the series of DNA fragments is provided by

- (i) contacting the template in the presence of DNA polymerase with a mixture of nucleotides for hybridising to the template for forming a second strand of DNA complementary to the template, wherein the mixture further comprises a set of four probes containing all four nucleotides for hybridising to the templates in which the nucleotide of each probe comprises a modified nucleotide which is capable of polymerising to the second strand of DNA but reversibly blocked to prevent further polymerisation thereto, wherein the step of contacting forms a series of templates containing all possible lengths of the second strand of DNA, each second strand terminated with one of the probes;
- (ii) removing unincorporated nucleotides;
- (iii) unblocking the modified nucleotides; and
- (iv) contacting the series of templates with an array of oligonucleotide probes,

Application No. 09/462,408
Attorney's Docket No. 020600-283
Page 8

wherein each oligonucleotide probe has a nucleotide sequence of common length 2 to 6, all combinations of nucleotide sequences are present in the array, and wherein each probe is cleavably attached to the mass label, which mass label is uniquely resolvable in mass spectrometry for identifying the nucleotide sequence.

Claim 10. (Previously Presented) A method according to claim 2, wherein the at least one template is a plurality of primed DNA single-stranded templates, each at a unique concentration, and the series of DNA fragments is provided by

- (i) contacting the templates in the presence of DNA polymerase with a mixture of nucleotides for hybridising to the template for forming a second strand of DNA complementary to the templates, wherein the mixture further comprises a set of four probes containing all four nucleotides for hybridising to the templates in which the nucleotide of each probe comprises a modified nucleotide which is capable of polymerising to the second strand of DNA but reversibly blocked to prevent further polymerisation thereto, wherein the step of contacting forms a series of templates containing all possible lengths of the second strand of DNA, each second strand terminated with one of the probes;
- (ii) removing unincorporated nucleotides;
- (iii) unblocking the modified nucleotides; and
- (iv) contacting the series of templates with an array of oligonucleotide probes, wherein each oligonucleotide probe has a nucleotide sequence of common length 2 to 6, all combinations of nucleotide sequences are present in the array, and wherein each probe is

Application No. 09/462,408
Attorney's Docket No. 020600-283
Page 9

cleavably attached to the mass label, which mass label is uniquely resolvable in mass spectrometry for identifying the nucleotide sequence.

Claim 11. (Previously Presented) A method according to claim 2, wherein the series of DNA fragments is provided by contacting the template in the presence of DNA ligase with a mixture of oligonucleotides for hybridising to the template for forming a second strand of DNA complementary to the template, the oligonucleotides each having a common length in the range 2 to 6, wherein the mixture further comprises a set of probes containing all possible oligonucleotides of the common length L for hybridising to the templates in which the oligonucleotide of each probe comprises a modified oligonucleotide which is capable of ligating to the second strand of DNA but blocked to prevent further ligation thereto and which is cleavable attached to the mass label, which mass label is uniquely resolvable in mass spectrometry for identifying the modified oligonucleotide, and the series of fragments contains all possible lengths of the second strand of DNA of integer multiples of L in which each fragment is terminated with one of the probes.

Claim 12. (Previously Presented) A method according to claim 2, wherein the at least one template is a plurality of primed DNA single-stranded templates, each at a unique concentration, and the series of DNA fragments is provided by contacting the templates in the presence of DNA ligase with a mixture of oligonucleotides for hybridising to the templates for forming a second strand of DNA complementary to the templates, the oligonucleotides

Application No. 09/462,408
Attorney's Docket No. 020600-283
Page 10

each having a common length in the range 2 to 6, wherein the mixture further comprises a set of probes containing all possible oligonucleotides of the common length L for hybridising to the templates in which the oligonucleotide of each probe comprises a modified oligonucleotide which is capable of ligating to the second strand of DNA but blocked to prevent further ligation thereto and which is cleavably attached to the mass label, which mass label is uniquely resolvable in mass spectrometry for identifying the modified oligonucleotide, and the series of fragments contains all possible lengths of the second strand of DNA of integer multiples of L in which each fragment is terminated with one of the probes.

Claim 13. (Previously Presented) A method according to claim 5, wherein the plurality of single-stranded templates is primed by hybridising to a known sub-sequence common to each of the templates an array of primers each comprising a base sequence containing a common sequence complementary to the known sub-sequence and a variable sequence of common length, in the range of 2 to 6, in which the array contains all possible nucleotide sequences of that common length and the mass label cleavably attached to each primer identifies the variable sequence, which variable sequence identifies the template to be sequenced.

Claim 14. (Original) A method according to claim 8, wherein the step of sorting the pooled fragments comprises contacting the fragments with an array of spatially separate

Application No. 09/462,408
Attorney's Docket No. 020600-283
Page 11

oligonucleotides each comprising a base sequence containing a common sequence complementary to the primer sequence of the fragments and a variable sequence of the common length, which array contains all possible variable sequences of the common length.

Claim 15. (Previously Presented) A method according to claim 4, wherein the reaction zones are separate containers.

Claim 16. (Previously Presented) A method according to claim 3, wherein the mixture of nucleotides comprises ATP, TTP, CTP and GTP.

Claim 17. (Previously Presented) A method according to claim 2, wherein the modified nucleotides are dideoxy- or deoxynucleotides.

Claim 18. (Previously Presented) A method according to claim 2, wherein the primed DNA is immobilised on a solid support.

Claim 19. (Canceled)

Claim 20. (Previously Presented) A method according to claim 1, wherein each mass label is cleavably attached to a fragment by a linker cleavable in a mass spectrometer.

Application No. 09/462,408
Attorney's Docket No. 020600-283
Page 12

Claim 21. (Previously Presented) A method for characterising DNA, which comprises

- (a) providing a primed DNA single-stranded template;
- (b) contacting the template in the presence of DNA polymerase with a mixture of nucleotides for hybridising to the template for forming a second strand of DNA complementary to the template, wherein the mixture further comprises a set of four probes containing all four nucleotides for hybridising to the templates in which the nucleotide of each probe comprises a modified nucleotide which is capable of polymerising to the second strand of DNA but reversibly blocked to prevent further polymerisation thereto, wherein the step of contacting forms a series of templates containing all possible lengths of the second strand of DNA, each second strand terminated with one of the probes;
- (c) removing unincorporated nucleotides;
- (d) unblocking the modified nucleotides;
- (e) contacting the series of templates with an array of oligonucleotide probes to form a series of fragments, each oligonucleotide probe having a nucleotide sequence of common length 2 to 6, and all combinations of sequences being present in the array, wherein each probe is cleavably attached to a mass label uniquely resolvable in mass spectrometry for identifying the nucleotide sequence;
- (f) separating the fragments by capillary electrophoresis, thereby determining the length of each fragment;
- (g) cleaving each fragment by collision to release its mass label; and

Application No. 09/462,408
Attorney's Docket No. 020600-283
Page 13

(h) determining each mass label by mass spectrometry to relate a nucleotide sequence that corresponds to the mass label to a position in the template so as to deduce the sequence of the template in order to characterise the DNA.

Claim 22. (Previously Presented) A method for characterising DNA, which comprises

- (a) providing a plurality of primed DNA single-stranded templates, each at a unique concentration;
- (b) contacting the templates in the presence of DNA polymerase with a mixture of nucleotides for hybridising to the template for forming a second strand of DNA complementary to the templates, wherein the mixture further comprises a set of four probes containing all four nucleotides for hybridising to the templates in which the nucleotide of each probe comprises a modified nucleotide which is capable of polymerising to the second strand of DNA but reversibly blocked to prevent further polymerisation thereto, wherein the step of contacting forms a series of templates containing all possible lengths of the second strand of DNA, each second strand terminated with one of the probes;
- (c) removing unincorporated nucleotides;
- (d) unblocking the modified nucleotides;
- (e) contacting the series of templates with an array of oligonucleotide probes to form a series of fragments, each oligonucleotide probe having a nucleotide sequence of common length 2 to 6, and all combinations of sequences being present in the array, wherein each

Application No. 09/462,408
Attorney's Docket No. 020600-283
Page 14

probe is cleavably attached to a mass label uniquely resolvable in mass spectrometry for identifying the nucleotide sequence;

- (f) separating the fragments by capillary electrophoresis, thereby determining the length of each fragment;
- (g) cleaving each fragment by collision to release its mass label; and
- (h) determining the identity and amount of each mass label by mass spectrometry to relate a nucleotide sequence of a probe that corresponds to the mass label to a position in its respective template so as to deduce the sequence of the template in order to characterise the DNA.

Claim 23. (Previously Presented) A method for characterising DNA, which comprises

- (a) providing a primed DNA single-stranded template;
- (b) contacting the template in the presence of DNA ligase with a mixture of oligonucleotides for hybridising to the template for forming a second strand of DNA complementary to the template, the oligonucleotides each having a common length in the range 2 to 6, wherein the mixture further comprises a set of probes containing all possible oligonucleotides of the common length L for hybridising to the templates in which the oligonucleotide of each probe comprises a modified oligonucleotide which is capable of ligating to the second strand of DNA but blocked to prevent further ligation thereto and which is cleavably attached to a corresponding mass label uniquely resolvable in mass

Application No. 09/462,408
Attorney's Docket No. 020600-283
Page 15

spectrometry for identifying the modified oligonucleotide, wherein the step of contacting forms a series of fragments containing all possible lengths of the second strand of DNA of integer multiples of L, each fragment terminated with one of the probes;

- (c) separating the fragments by capillary electrophoresis, thereby determining the length of each fragment;
- (d) cleaving each fragment by collision to release its mass label; and
- (e) determining each mass label by mass spectrometry to relate its corresponding oligonucleotide to a position in the template so as to deduce the sequence of the template in order to characterise the DNA.

Claim 24. (Previously Presented) A method for characterising DNA, which comprises

- (a) providing a plurality of primed DNA single-stranded templates, each at a unique concentration;
- (b) contacting the templates in the presence of DNA ligase with a mixture of oligonucleotides for hybridising to the templates for forming a second strand of DNA complementary to the templates, the oligonucleotides each having a common length in the range 2 to 6, wherein the mixture further comprises a set of probes containing all possible oligonucleotides of the common length L for hybridising to the templates in which the oligonucleotide of each probe comprises a modified oligonucleotide which is capable of ligating to the second strand of DNA but blocked to prevent further ligation thereto and

Application No. 09/462,408
Attorney's Docket No. 020600-283
Page 16

which is cleavably attached to a corresponding mass label uniquely resolvable in mass spectrometry for identifying the modified oligonucleotide, wherein the step of contacting forms a series of fragments containing all possible lengths of the second strand of DNA of integer multiples of L, each fragment terminated with one of the probes;

- (c) separating the fragments by capillary electrophoresis, thereby determining the length of each fragment;
- (d) cleaving each fragment by collision to release its mass label; and
- (e) determining the identity and amount of each mass label by mass spectrometry to relate its corresponding oligonucleotide to a position in its respective template so as to deduce the sequence of the template in order to characterise the DNA.

Claims 25-26. (Canceled)

Claim 27. (Previously Presented) A method for characterizing DNA, which comprises:

- (a) providing at least one DNA single-stranded template primed with a primer;
- (b) generating a population of fragments of said DNA from the at least one template by contacting the at least one template in the presence of DNA polymerase with a mixture of nucleotides for hybridising to the at least one template for forming a second strand of DNA complementary to the at least one template, wherein the mixture further comprises a set of four probes containing all four nucleotides for hybridising to the at least one template in

Application No. 09/462,408
Attorney's Docket No. 020600-283
Page 17

which the nucleotide of each probe comprises a modified nucleotide or oligonucleotide which is capable of polymerising to the second strand of DNA but blocked to prevent further polymerisation thereto, which modified nucleotide or oligonucleotide is cleavably attached to the mass label for identifying the modified nucleotide or oligonucleotide, which mass label is cleavable from the probe in a mass spectrometer and is resolvable by mass spectrometry, and wherein each fragment is terminated with one of the probes, wherein the population comprises at least one series of DNA fragments, the series containing all possible lengths of a second strand of DNA complementary to the or each template;

- (c) separating the fragments by capillary electrophoresis, thereby determining the length of each fragment;
- (d) cleaving each fragment in a mass spectrometer by collision to release its mass label; and
- (e) determining each mass label by mass spectrometry to identify a terminating modified nucleotide or oligonucleotide of each fragment by the length of the fragment in order to characterize said DNA.

Claim 28. (Previously Presented) A method for characterizing DNA, which comprises:

- (a) providing at least one strand of the DNA as a single-stranded template primed with a set of oligonucleotide primers, each of which primers comprises a mass label cleavably attached to an oligonucleotide primer base sequence for hybridising to a single-stranded DNA

Application No. 09/462,408
Attorney's Docket No. 020600-283
Page 18

template to form a primed template, wherin each mass label is cleavable from the primer in a mass spectrometer by collision, uniquely resolvable in relation to every other mass labels in the set by mass spectrometry and identifies the oligonucleotide primer base sequence; and

(b) generating a population of fragments of said DNA from the and each template by contacting the and each template in the presence of DNA polymerase with a mixture of nucleotides for hybridising to the and each template for forming a second strand of DNA complementary to the and each template, wherein the population comprises at least one series of DNA fragments, the series containing all possible lengths of a second strand of DNA complementary to the or each template;
wherein a feature of each fragment identified by each mass label relates to a nucleotide or nucleotide sequence at one end of each fragment, so that each nucleotide corresponds to a position in the template primed with the primers comprising the mass label so as to deduce the sequence of the or each template in order to characterise the DNA.